Supplemental Document

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Highly sensitive detection of cytochrome c in the NSCLC serum using a hydrophobic paper based–gold nanourchin substrate: supplement

YUE SUN,[1,2] SHENGJIE GE,[1,2] JIN XUE,[3] XINYU ZHOU,[1,2] WENBO LU,[4] GUANG LI,[5] AND XIAOWEI CAO[1,2,6,*]

[1]*Institute of Translational Medicine, Medical College, Yangzhou University, Yangzhou 225001, China*
[2]*Jiangsu Key Laboratory of Experimental and Translational Non-coding RNA Research, Medical College, Yangzhou University, Yangzhou 225001, China*
[3]*Guangling College, Yangzhou University, Yangzhou 225001, China*
[4]*Shanxi Normal University, College of Chemistry and Material Science, Linfen 041004, China*
[5]*Department of Otorhinolaryngology, Affiliated Hospital of Yangzhou University, Yangzhou 225001, China*
[6]*Jiangsu Key Laboratory of Zoonosis, Yangzhou University, Yangzhou 225009, China*
[*]*cxw19861121@163.com*

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Supplementary materials

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YUE SUN,[1,6] SHENGJIE GE,[1,6] JIN XUE,[2] XINYU ZHOU,[1,6] WENBO LU,[5] GUANG LI,[4] XIAOWEI CAO[1,3,6,*]

[1]*Institute of Translational Medicine, Medical College, Yangzhou University, Yangzhou, 225001, P. R. China*

[2]*Guangling College, Yangzhou University, Yangzhou, 225001, PR China*

[3]*Jiangsu Key Laboratory of Zoonosis, Yangzhou University, Yangzhou, 225009, P. R. China*

[4]*Department of otorhinolaryngology, Affiliated Hospital of Yangzhou University, Yangzhou, 225001, P. R. China*

[5]*Shanxi Normal University, College of Chemistry and Material Science, Linfen, 041004, P. R. China*

[6]*Jiangsu Key Laboratory of Experimental & Translational Non-coding RNA Research, Medical College, Yangzhou University, Yangzhou, 225001, P. R. China*

[*]*E-mail: cxw19861121@163.com*

Optimization of experimental conditions

Optimization the concentration of of Cyt c aptamer conjugated to GNUs

It was well known that the aqueous Cyt c aptamer solution, as a kind of DNA, had a typical absorption peak (about 260 nm) [1,2]. The spectroscopy of functionalized GNUs substrate was investigated using a Hitachi U-3000 UV-visible spectrophotometer at a scan rate of 600 nm/min. After the attachment of Cyt c aptamer to GNUs, the amount of free Cyt c on the surface of GNUs substrate decreased and the absorption intensity weakened (Fig. S1). As a result, the decrease of this value can be used to certify the GNUs were modified by DNA. The amount of Cyt c aptamer was optimized using this value of the UV-visible absorption peak at 260 nm. Different concentration (100 nM, 250 nM, 500 nM, 800 nM, 1000 nM and 1200 nM) of 50 μL Cyt c aptamer were added in the functionalization of SERS substrates. With the concentration of Cyt c aptamer increased, the absorption intensity at 260 nm decreased significantly until the concentration reached 1000 nM (Fig. S2A). Thus, the Cyt c aptamer concentration of 1000 nM was chosen for the subsequent investigations.

Optimization of concentration of Cy5-labeled complementary DNA

In Fig. S2B, the concentration of Cy5-labeled complementary DNA was also optimized to obtain the highest initial signal. When Cy5-labeled complementary DNA was connected with Cyt c aptamer-modified GNUs, the strong SERS signal of Cy5 was generated. Thus, the concentration of Cy5-labeled complementary DNA was optimized using the SERS intensity of Cy5 SERS characteristic peak at 1360 cm^{-1} (as shown in Fig. S2C). Different concentrations (50 nM, 100 nM, 200 nM, 500 nM and 1000 nM)

of 50 μL Cy5-labeled complementary DNA were incubated with the Cyt c aptamer- modified GNUs substrate, respectively. With the increased concentration from 50 to 1000 nM, the SERS intensity at 1360 cm^{-1} increased sharply until the concentration reached 500 nM. It can be inferred that the connection of Cy5-labeled complementary DNA reached saturation when the concentration was 500 nM. Hence 500 nM was selected as the optimal concentration of Cy5-labeled complementary DNA.

Optimization of hybridization time for the reaction between Cy5-labeled complementary DNA and Cyt c aptamer

Fig. S2D presented the optimization of hybridization time for the reaction between Cyt c aptamer and Cy5-labeled complementary DNA. The hybridization time was closely related to the SERS enhancement. 50 μL of 500 nM Cy5-labeled complementary DNA were incubated with the Cyt c aptamer-modified GNUs substrate for 10 min, 20 min, 30 min, 40 min, 50 min, 60 min and 70 min at 37 °C, respectively. In Fig. S2D, the SERS signal intensity increased with hybridization time from 10 to 50 min and then plateaued. Therefore, the optimal hybridization time was 50 min.

Optimization of incubation time of aptamer bonding to Cyt c

Besides, incubation time of aptamer bonding to Cyt c was optimized in this study, which is an important factor for biosensor evaluation. 10 μL of 10 μg/mL Cyt c solution was added to the surface of the above functionalized substrates which were prepared under the optimal experimental conditions. Different incubation times (30 min, 60 min, 90 min, 120 min, 150 min, 180 min and 210 min) were studied at 37 °C. As shown in Fig. S2E, specific recognition between Cyt c and DNA aptamer can be completed within 120 min. Thus, 120 min was the optimal incubation time for subsequent experiments.

Optimization of pH values

In Fig. S2F, the specific binding ability between Cyt c aptamer and Cyt c at different pH values was also investigated. A lower SERS signal indicated that more Cyt c aptamers were specifically bound by the Cyt c molecules. After 10 μL of 10 μg/mL Cyt c solution was added to the surface of the above functionalized substrates, different pH values (7.0, 7.2, 7.4, 7.6, 7.8) were studied at 37 °C. It can be obtained from the results that the specific binding ability between Cyt c aptamer and Cyt c was the lowest at pH 7.4 and pH 7.4 was chosen as the optimal incubation pH.

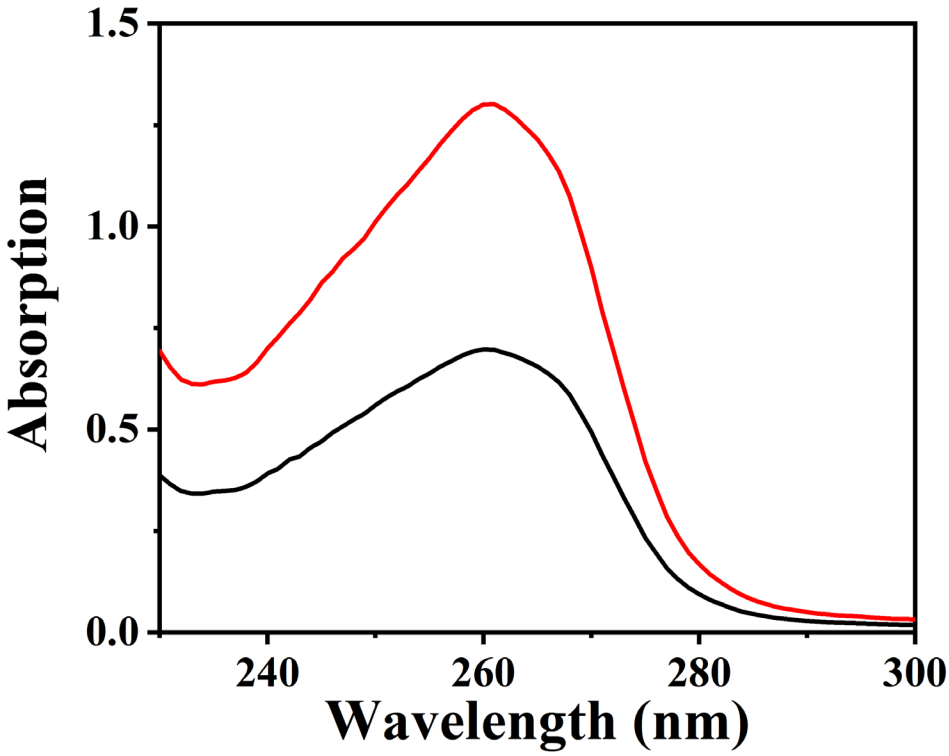

Fig. S1. UV-visible absorption spectra of the aqueous Cyt c aptamer solution (red) and the Cyt c aptamer-labeled GNUs substrate (black).

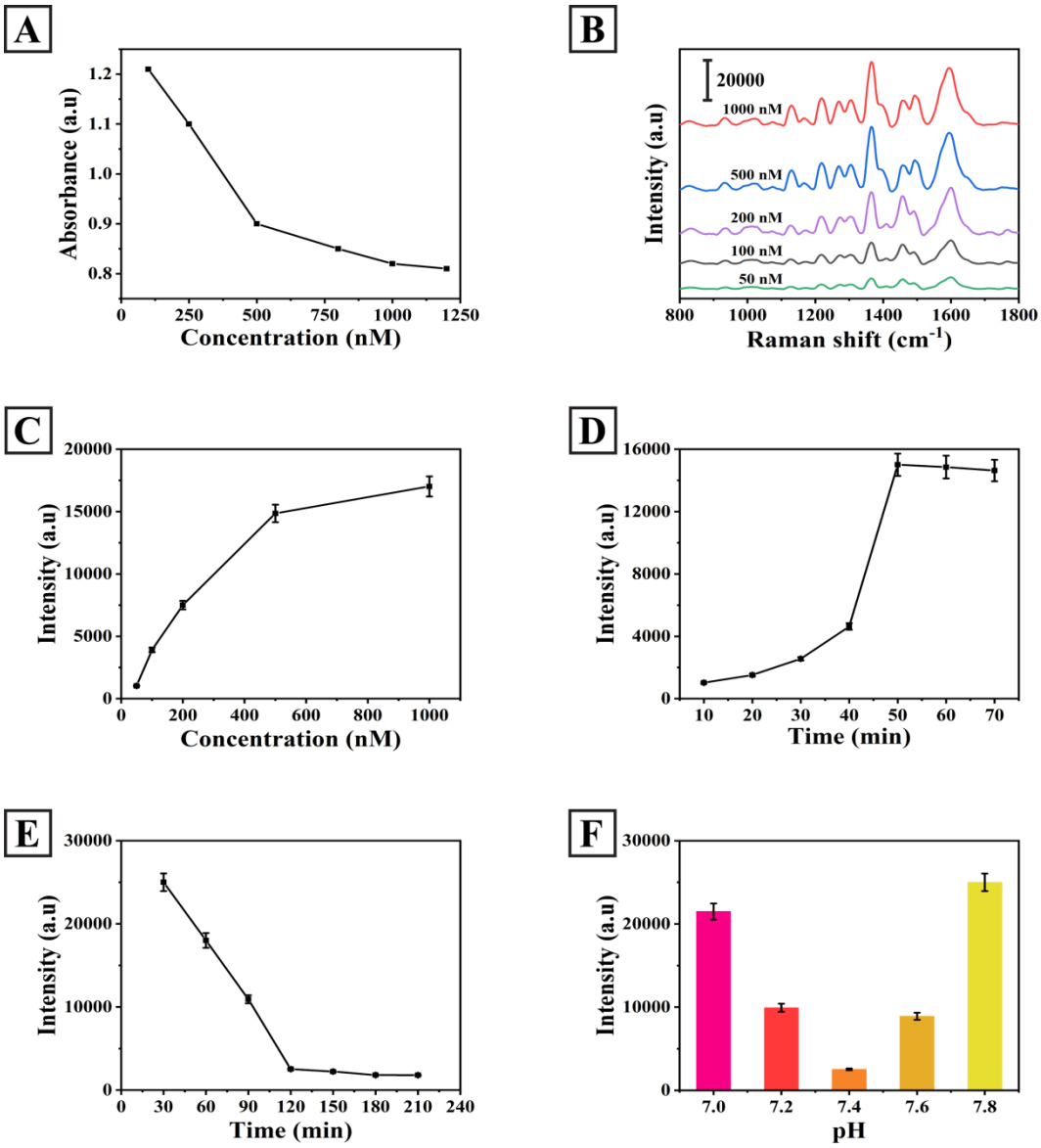

Fig. S2. (A) Optimization of Cyt c aptamer. (B) Optimization of Cy5-labeled complementary DNA. (C) SERS intensities of Cy5 SERS characteristic peak at 1360 cm^{-1} corresponding to B. (D) Optimization of hybridization time for the reaction between Cyt c aptamer and Cy5-labeled complementary DNA. (E) Optimization of incubation time of aptamer bonding to Cyt c. (F) Comparison of specific binding capacity of Cyt c aptamer and Cyt c at different pH values. Error bars indicated standard deviation of three replicate determinations.

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